09/578 507
#1 #2

from a pellet of the centrifuged microorganisms, and the supernatant gives wild-type BDNF and
the extract gives 3 types of BDNF having biological activities (in terms of ED50) of 2, 9 and
15 ng/ml respectively. Use of a signal sequence from E. coli permits BDNF to be produced in
periplasm of E. coli.

L11: Entry 27 of 28

File: DWPI

Jun 25, 1996

DERWENT-ACC-NO: 1996-348981
DERWENT-WEEK: 199635
COPYRIGHT 2001 DERWENT INFORMATION LTD

TITLE: Purification of brain-derived nerve nutrition factor used e.g. in drugs - from supernatant
obtd. by disruption and centrifugation of genetic recombinant E. coli

ABTX:
The purificn. includes ion-exchange chromatography, gel filtration and reverse phase chromatography.
The BDNF-producing E. coli carries a plasmid contg. a BDNF gene linked to a single peptide gene for
beta-lactamase. Disulphide linkages in BDNF purified from recombinant E. coli are e.g.
Cys(5)-Cys(68), Cys(80)-Cys(109), Cys(13)-Cys(111).

28. Document ID: JP 04234988 A

L11: Entry 28 of 28

File: DWPI

Aug 24, 1992

DERWENT-ACC-NO: 1992-327632
DERWENT-WEEK: 199240
COPYRIGHT 2001 DERWENT INFORMATION LTD

TITLE: Human prolactin and its protein A fused protein expression gene - for treatment and diagnosis
of pituitary tumours and sterility

PRIORITY-DATA: 1991JP-0012506 (January 11, 1991)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 04234988 A	August 24, 1992	N/A	008	C12N015/62

INT-CL (IPC): A61B 10/00; A61K 37/32; C07K 13/00; C12N 15/62; C12P 21/02; G01N 33/53; C12P 21/02; C12R 1/19

AB: Fused protein expression gene has a gene coding collagenase (III) cleavage site of
amino acid sequence (1) (Gly-Xaa-Gly-Pro-Xaa) between human prolactin (I) gene and a gene
coding the region (PA) contg. at least Fc-bond active peptide fragment of a protein A (II).,

USE/ADVANTAGE - Used in pharmaceuticals for diagnosis of pituitary tumour and sterility., In an
example, cDNA of (I) is prepd. (I) gene is cloned. One of 6 clones is digested by EcoRI and
recombined to the EcoRI site of plasmid vector pUC9 to give cDNA cloning vector of (I). It is
introduced to E. coli and amplified. DNA coding (III) linker is prepd. PA-(III)
linker-(I)-fused protein expression vector is constructed. PA(III) linker-(I)-fused protein
expression vector, pRIT-LK-PL1.2, is expressed. (I) is purified from the fused protein by a
Sepharose 4B column, a HPLC and a reversed phase chromatography. The cleavage condition of the
pure fused protein by (III) is examined. (I) shows a same immunological reactivity as
commercial (I). It shows no crossing over with other hormones such as human growth hormone and
human placental lactogen as to an antibody prepd. by using the fused protein as the antigen

L11: Entry 28 of 28

File: DWPI

Aug 24, 1992

DERWENT-ACC-NO: 1992-327632
DERWENT-WEEK: 199240
COPYRIGHT 2001 DERWENT INFORMATION LTD

TITLE: Human prolactin and its protein A fused protein expression gene - for treatment and diagnosis
of pituitary tumours and sterility

ABTX:
In an example, cDNA of (I) is prepd. (I) gene is cloned. One of 6 clones is digested by EcoRI and
recombined to the EcoRI site of plasmid vector pUC9 to give cDNA cloning vector of (I). It is
introduced to E. coli and amplified. DNA coding (III) linker is prepd. PA-(III) linker-(I)-fused
protein expression vector is constructed. PA(III) linker-(I)-fused protein expression vector,
pRIT-LK-PL1.2, is expressed. (I) is purified from the fused protein by a Sepharose 4B column, a HPLC
and a reversed phase chromatography. The cleavage condition of the pure fused protein by (III) is
examined. (I) shows a same immunological reactivity as commercial (I). It shows no crossing over
with other hormones such as human growth hormone and human placental lactogen as to an antibody
prepd. by using the fused protein as the antigen

Terms	Documents
l9 same l1	28

Documents, starting with Document: 1.

Document ID: US 6197553 B1

L13: Entry 1 of 2

File: USPT

Mar 6, 2001

US-PAT-NO: 6197553
DOCUMENT-IDENTIFIER: US 6197553 B1
TITLE: Method for large scale plasmid purification

DATE-ISSUED: March 6, 2001

US-CL-CURRENT: 435/91.1; 424/184.1, 435/259, 435/306.1, 435/320.1, 514/44, 536/23.1, 536/25.4

APPL-NO: 8/ 952428
DATE FILED: November 7, 1997

PARENT-CASE:
RELATED APPLICATION This is a 35 U.S.C. .sctn.371 U.S. national application of PCT/US96/07083, filed
May 15, 1996, which is a continuation-in-part of U.S. application Ser. No. 08/446,118, filed May 19,
1995, now abandoned, which is a continuation-in-part of U.S. application Ser. No. 08/275,571, filed
Jul. 15, 1994, now abandoned.

IN: Lee; Ann L, Sagar; Sangeetha

AB: A process is disclosed for the large scale isolation and purification of plasmid DNA
from large scale microbial fermentations. The process exploits a rapid heating method to induce
cell lysis and precipitate genomic DNA, proteins and other debris while keeping the plasmid in
solution. Suspending the microbial cells in buffer and then heating the suspension to about
70-100.degree. C. in a flow-through heat exchanger results in excellent lysis. Continuous flow
or batch-wise centrifugation of the lysate effects a pellet that contains the cell debris,
protein and most of the genomic DNA while the plasmid remains in the supernatant. This
invention offers a number of advantages including higher product recovery than by chemical
lyses, inactivation of Dnases, operational simplicity and scaleability.

L13: Entry 1 of 2

File: USPT

Mar 6, 2001

DOCUMENT-IDENTIFIER: US 6197553 B1
TITLE: Method for large scale plasmid purification

DEPR:
Isolation of the plasmid DNA from harvested microbial cells using the current lab scale procedures
consist mainly of enzymatic treatment of microbial cells to weaken the cell wall followed by cell
lysis. The purification steps include repetitive CsCl/EtBr centrifugations followed by organic
solvent extractions and precipitation to remove tRNA, residual proteins, EtBr and other host
contaminants. These steps are not scaleable and therefore not suitable for use in large-scale
processing. In contrast, preparative scale chromatography is a powerful purification tool that
provides high resolution, operational ease and increased productivity for purifying DNA plasmid
products. Two different modes of chromatography, reversed phase and anion exchange, show suitability
in purifying DNA plasmid to the stringent levels required for human use. Separations based on
reversed phase are governed by hydrophobic interactions while those for anion exchange are based on
electrostatic interaction. These two orthogonal chromatography steps achieve separations between
various forms of plasmid (supercoiled, open relaxed, linear and concatemers) and remove host
contaminants like LPS (endotoxin), RNA, DNA and residual proteins.

DEPR:
The anion exchange product was then loaded onto a reversed phase chromatography column (Poros R/H)
which had been previously equilibrated with 100 mM ammonium bicarbonate at pH 8.0, and a gradient of
0% to 80% methanol was used to elute the bound material. The highly purified supercoiled plasmid DNA
eluted at 22% methanol.

2. Document ID: US 5561064 A

L13: Entry 2 of 2

File: USPT

Oct 1, 1996

US-PAT-NO: 5561064
DOCUMENT-IDENTIFIER: US 5561064 A
TITLE: Production of pharmaceutical-grade plasmid DNA
DATE-ISSUED: October 1, 1996

US-CL-CURRENT: 435/320.1; 435/259, 435/91.1

APPL-NO: 8/ 192151
DATE FILED: February 1, 1994

IN: Marquet; Magda, Horn; Nancy, Meek; Jennifer, Budahazi; Gregg

AB: The invention relates to a method for producing plasmid DNA, comprising the steps
of: (a) lysing cells containing the plasmid DNA to obtain a lysate; (b) treating the lysate by
a means for removing insoluble material to obtain a solute; and (c) applying the solute to
differential PEG precipitations and chromatography to purify the plasmid DNA. In other
embodiments of the invention, the plasmid DNA is produced with GRAS reagents; the plasmid DNA
is produced in the absence of enzymes; the plasmid DNA is produced in the absence of organic
extractants; the plasmid DNA is produced in the absence of mutagens; the lysing, treating and
applying steps are scalable to result in the large scale manufacture of the plasmid DNA; and
the lysing, treating and applying steps result in the generation of pharmaceutical grade
material.

L13: Entry 2 of 2

File: USPT

Oct 1, 1996

DOCUMENT-IDENTIFIER: US 5561064 A
TITLE: Production of pharmaceutical-grade plasmid DNA

DEPR:
Upon being separated from many host contaminants, such as chromosomal DNA, RNA, lipopolysaccharide
and protein, a sample is obtained that is rich in plasmid DNA and yet may harbor small RNA
oligonucleotides, trace amounts of chromosomal DNA, protein, endotoxins and residues left over from
processing. According to the invention, further purification may be effected as an independent step
to rid product of remaining nucleic acids, macromolecules, small molecular forms and residuals, and,
moreover, to isolate covalently closed circular DNA, i.e., supercoiled monomers, from nicked
circular plasmids (relaxed monomers) and concatenated forms (supercoiled dimers, etc.). Towards this
end, a chromatography step is performed. Differences in ionic charge,

molecular size, and/or other
characteristics are exploited to bring about purification of the desired plasmid DNA species.
Chromatography is contemplated to encompass ion exchange chromatography, size exclusion
chromatography, reversed phase chromatography, hydrophobicity interaction chromatography, affinity
chromatography, and any like chromatography, and, also, any combination of these to bring about the
final purification of plasmid DNA.

1. Document ID: US 20010007756 A1

L11: Entry 1 of 28

File: PGPB

Jul 12, 2001

PGPUB-DOCUMENT-NUMBER: 20010007756
PGPUB-FILING-TYPE: new-utility
DOCUMENT-IDENTIFIER: US 20010007756 A1

TITLE: COSMETIC COMPOSITIONS

PUBLICATION-DATE: July 12, 2001
US-CL-CURRENT: 435/69.1; 435/320.1, 514/773, 524/401, 530/323

APPL-NO: 09/037193
DATE FILED: March 9, 1998
CONTINUED PROSECUTION APPLICATION: CPA

RELATED-US-APPL-DATA:
RLAN
RLFD
RLPC
RLKC
RLAC
09037193
Mar 9, 1998
GRANTED
A1
US
08641627
May 2, 1996
US
5726040

IN: ENSLEY, BURT D.

AB: A cosmetic composition including a non naturally-occurring extracellular matrix
protein in combination with a cosmetic carrier is described. The protein is preferably of human
origin and has not been previously cross-linked. The protein is most preferably selected from
the group consisting of soluble human procollagen and soluble human tropoelastin. Preferably,
the composition contains at least two allelic variants of the protein, most preferably in
substantially the same ratio at which they are found in epidermis of a selected individual. The
individual may be selected, for example, on the basis of having youthful-appearing skin, of
being the future wearer of the composition, or of other reasons.

L11: Entry 1 of 28

File: PGPB

Jul 12, 2001

DOCUMENT-IDENTIFIER: US 20010007756 A1
TITLE: COSMETIC COMPOSITIONS

DETX:
[0202] The tropoelastin cDNA, containing the synthetic oligonucleotides in reading frame, cloned
into, for example, pAS-MCS72, is transformed into the lysogenic host E. coli AR120, and
transformants are selected using routine procedures. Bacteria bearing the expression plasmid induced
by, for example, 60 mg/ml nalidixic acid, are shaken at 37.degree. C. to allow for expression of the
tropoelastin isomorph. Bacterial pellets are suspended in buffer, treated with lysozyme and then
centrifuged. The pellet from the lysozyme treatment is suspended in buffer, homogenized, and
centrifuged. The pellets, containing tropoelastin associated with the cell membranes, is treated
with CNBr, releasing solubilized, intact tropoelastin fusion protein. Additional purification is
achieved using reverse phase chromatography, or other method.

2. Document ID: US 20010007026 A1

L11: Entry 2 of 28

File: PGPB

Jul 5, 2001

PGPUB-DOCUMENT-NUMBER: 20010007026
PGPUB-FILING-TYPE: new-utility
DOCUMENT-IDENTIFIER: US 20010007026 A1

TITLE: METHOD OF SEPARATING NUCLEIC ACIDS BY MEANS OF LIQUID CHROMATOGRAPHY

PUBLICATION-DATE: July 5, 2001
US-CL-CURRENT: 536/25.4

APPL-NO: 09/309599
DATE FILED: May 11, 1999
CONTINUED PROSECUTION APPLICATION: CPA

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY
APPL-NO
DOC-ID
APPL-DATE
JP
10-127665
1998JP-10-127665
May 11, 1998

IN: KITAMURA, TAKASHI, NAKATANI, SHIGERU

AB: Disclosed is a method for separating nucleic acids by hydrophobic interaction
chromatography. The purpose is to provide a method for separating and purifying nucleic acids
by hydrophobic interaction chromatography, which enables to separate nucleic acids such as
plasmids and DNA fragments in a shorter time.

L11: Entry 2 of 28

File: PGPB

Jul 5, 2001

DOCUMENT-IDENTIFIER: US 20010007026 A1
TITLE: METHOD OF SEPARATING NUCLEIC ACIDS BY MEANS OF LIQUID CHROMATOGRAPHY

BSTX:
[0011] For solving the problems in chemical separating and purifying method and electrophoretic
separation as explained above, a method of separating and purifying nucleic acids that utilizes
liquid chromatography has been used recently. So far, there are examples, wherein long chain nucleic
acids such as plasmids were separated and purified by using ion exchange chromatography and reversed
phase chromatography.

3. Document ID: US 6287559 B1

L11: Entry 3 of 28

File: USPT

Sep 11, 2001

US-PAT-NO: 6287559
DOCUMENT-IDENTIFIER: US 6287559 B1
TITLE: Cloning and recombinant production of vespid venom hyaluronidases, and immunological
therapies based thereon
DATE-ISSUED: September 11, 2001

US-CL-CURRENT: 424/94.62; 435/201, 435/252.3, 435/320.1, 435/69.1, 530/350, 536/23.2, 536/23.4,
536/23.5

APPL-NO: 8/ 474853
DATE FILED: June 7, 1995

PARENT-CASE:
The present invention is a Division of application Ser. No. 08/180,209 filed Jan. 11, 1994, now U.S.
Pat. No. 5,593,877 which is a continuation-in part of application Ser. No. 08/031,400, filed Mar.
11, 1993, now abandoned, of which the instant Application claims the benefit of the filing date
under 35 U.S.C. .sctn. 120, and the disclosure of which is incorporated herein by reference in its
entirety.

IN: King; Te Piao

AB: The present invention is directed to nucleic acids encoding vespid venom enzymes, or
fragments thereof, recombinant vectors comprising such nucleic acids, and host cells containing
the recombinant vectors. The invention is further directed to expression of such nucleic acids
to produce recombinant vespid venom enzymes, or recombinant fragments, derivatives or analogs
thereof. Such recombinant products are useful for diagnosis of allergy and for therapeutic
treatment of allergy. In specific embodiments, the present invention provides nucleic acids
encoding, and complete nucleotide and amino acids sequences for, vespid venom phospholipase,
for example, Dolichovespula maculata phospholipase and Vespula vulgaris phospholipase, and
vespid venom hyaluronidase, for example, Dolichovespula maculata hyaluronidase.

L11: Entry 3 of 28

File: USPT

Sep 11, 2001

DOCUMENT-IDENTIFIER: US 6287559 B1
TITLE: Cloning and recombinant production of vespid venom hyaluronidases, and immunological
therapies based thereon

DEPR:
The pQE12 plasmid is designed so that the recombinant protein has the sequence:
MRGS-insert-SRH.sub.6. The presence of the hexa-histidine sequence in the recombinant protein makes
possible its purification from other bacterial proteins by metal ion chelation chromatography
followed by reversed phase chromatography.

4. Document ID: US 6287554 B1

L11: Entry 4 of 28

File: USPT

Sep 11, 2001

US-PAT-NO: 6287554
DOCUMENT-IDENTIFIER: US 6287554 B1
TITLE: Chicken interleukin-15 and uses thereof
DATE-ISSUED: September 11, 2001

US-CL-CURRENT: 424/85.2; 424/278.1, 424/85.1, 530/324, 530/350, 530/351

APPL-NO: 9/ 368613
DATE FILED: August 4, 1999

PARENT-CASE:
This is a division of application Ser. No. 08/729,004, filed Oct. 10, 1996 now U.S. Pat. No.
6,190,901. This application claims the priority of provisional application Ser. No. 60/005,682 filed
Oct. 17, 1995. Each of these prior applications is hereby incorporated herein by reference, in its
entirety.

IN: Sundick; Roy S., Jones; Lily A., Smith; David I.

AB: The present invention pertains to isolated DNA encoding avian interleukin-15 and to
purified interleukin-15 polypeptides.

L11: Entry 4 of 28

File: USPT

Sep 11, 2001

DOCUMENT-IDENTIFIER: US 6287554 B1
TITLE: Chicken interleukin-15 and uses thereof

DEPR:
Purification of IL-15 from natural or recombinant sources may be achieved by methods well-known in
the art, including without limitation ion-exchange chromatography, reverse-phase chromatography on

C4 columns, gel filtration, isoelectric focusing, affinity chromatography, immunoaffinity
chromatography, and the like. In a preferred embodiment, large quantities of bioactive IL-15 may be
obtained by constructing a recombinant DNA sequence comprising the coding region for IL-15 fused in
frame to a sequence encoding 6 C-terminal histidine residues in the pSFV1 replicon (GIBCO/BRL). mRNA
encoded by this plasmid is synthesized using techniques well-known to those skilled in the art and
introduced into BHK-21 cells by electroporation. The cells synthesize and secrete mature
glycosylated IL-15 polypeptides containing 6 C-terminal histidines. The modified IL-15 polypeptides
are easily purified from the cell supernatant by affinity chromatography using a histidine-binding
resin (His-bind, Novagen, Madison, Wis.).

5. Document ID: US 6265168 B1

L11: Entry 5 of 28

File: USPT

Jul 24, 2001

US-PAT-NO: 6265168
DOCUMENT-IDENTIFIER: US 6265168 B1
TITLE: Apparatus and method for separating and purifying polynucleotides
DATE-ISSUED: July 24, 2001

US-CL-CURRENT: 435/6; 210/635, 435/1.3, 435/91.1, 435/91.2, 536/25.32, 536/25.4

APPL-NO: 9/ 318407
DATE FILED: May 25, 1999

PARENT-CASE:
RELATIONSHIP TO COPENDING APPLICATIONS This application is a regular U.S. patent application under
35 U.S.C. .sctn.111 (a) and 35 U.S.C. .sctn.1.53(b) and claims priority from the following
co-pending, commonly assigned U.S. Provisional Applications, each filed under 35 U.S.C. .sctn.111
(b): Ser. No. 60/103,313, filed Oct. 6, 1998; Ser. No. 60/117,211 filed Jan. 25, 1999; Ser. No.
60/117,178 filed Jan. 25, 1999; Ser. No. 60/119,945 filed Feb. 12, 1999; Ser. No. 60/123,301 filed
Mar. 3, 1999; Ser. No. 60/129,838 filed Apr. 16, 1999; Ser. No. 60/130,700 filed Apr. 23, 1999; and
the following co-pending commonly assigned non-provisional U.S. patent applications, each filed
under 53 U.S.C. .sctn.111: Ser. No. 09/039,061 filed Mar. 13, 1998 (pending); Ser. No. 09/058,337
filed Apr. 10, 1998 (abandoned); Ser. No. 09/058,580 filed Apr. 10, 1998 (abandoned); Ser. No.
09/081,039 filed May 18, 1998 (now U.S. Pat. No. 5,972,222); Ser. No. 09/129,105 filed Aug. 4, 1998
(now U.S. Pat. No. 6,024,878); Ser. No. 09/183,047 filed Qct. 30, 1998 (now U.S. Pat. No.
6,066,258); Ser. No. 09/183,123 filed Oct. 30, 1998 (now U.S. Pat. No. 6,056,877); Ser. No.
09/183,450 filed Oct. 30, 1998 (now U.S. Pat. No. 5,997,742); Ser. No. 09/183,573 filed Oct. 30,
1998 (abandoned); and Ser. No. 09/311,116 filed May 13, 1999. The entire contents of the
above-listed pending patent applications are hereby incorporated by reference.

IN: Gjerde; Douglas T., Hanna; Christopher P., Taylor; Paul D., Legendre, Jr.; Benjamin
L., Haefele; Robert M.

AB: A method for removing a target DNA fragment having a predetermined base-pair length
from a mixture of DNA fragments comprises the following steps. A mixture of DNA fragments which
may contain the target DNA fragments is applied to a separation column containing media having
a nonpolar, nonporous surface, the mixture of DNA fragments being in a first solvent mixture
containing a counterion and a DNA binding concentration of driving solvent in a cosolvent. The
target DNA fragments are separated from the media by contacting it with a second solvent
solution containing a counterion and a concentration of driving solvent in cosolvent which has
been predetermined to remove DNA fragments having the target DNA fragment base pair length from
the media. The target DNA fragments can be collected and optionally amplified. When the method
is being applied to collect a putative fragment, if present, no DNA fragments having the base
pair length of the target DNA could be present in the mixture. Alternatively, DNA fragments
having the base pair length of the target DNA are present in the mixture. The disclosure also
describes an ambient or low pressure device for separating polynucleotide fragments from a
mixture of polynucleotide fragments comprises a tube having an upper solution input chamber, a
lower eluant receiving chamber, and a fixed unit of separation media supported therein. The
separation media has nonpolar separation surfaces which are free from multivalent cations which
would react with counterion to form an insoluble polar coating on the surface of the separation
media.

L11: Entry 5 of 28

File: USPT

Jul 24, 2001

DOCUMENT-IDENTIFIER: US 6265168 B1
TITLE: Apparatus and method for separating and purifying polynucleotides

BSPR:
A need exists for rapid and efficient procedures for isolating, separating and purifying
single-stranded oligonucleotides and single-stranded DNA fragments, RNA single-stranded DNA
fragments, plasmids and the like. Traditional methods such as ion exchange chromatography, high
pressure reverse phase chromatography, gel electrophoresis, capillary electrophoresis and the like
are slow, laborious and inefficient, and they require the services of a highly skilled
chromatographic expert. Furthermore, many methods are incapable of effecting a base-pair length size
based separation of these fragments and are capable of yielding only minute quantities of separated
materials.

6. Document ID: US 6254870 B1

L11: Entry 6 of 28

File: USPT

Jul 3, 2001

US-PAT-NO: 6254870
DOCUMENT-IDENTIFIER: US 6254870 B1

TITLE: Thrombopoietin: IL-3 fusion protein
DATE-ISSUED: July 3, 2001

US-CL-CURRENT: 424/192.1; 424/198.1, 424/85.1, 424/85.2, 435/69.7, 530/351, 536/23.1

APPL-NO: 8/ 875533
DATE FILED: January 30, 1998

PARENT-CASE:
This is a continuation-in-part of U.S. application Ser. No. 08/383,035 filed Feb. 3, 1995, now
abandoned, which is incorporated herein by reference.

PCT-DATA:

APPL-NO	DATE-FILED	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
PCT/US96/00830	February 1, 1996	WO96/23888	Aug 8, 1996	Jan 30, 1998	Jan 30, 1998

IN: Staten; Nicholas R., Favara; Jean P., Kahn; Larry E., Pegg; Lyle E., McKearn; John
P., Baum; Charles M.

AB: The present invention relates to variants of human c-mpl ligands (thrombopoietin)
with activity on hematopoietic differentiation and expansion.

L11: Entry 6 of 28
File: USPT
Jul 3, 2001

DOCUMENT-IDENTIFIER: US 6254870 B1
TITLE: Thrombopoietin: IL-3 fusion protein

BSPR:
E. coli strain MON105 harboring the plasmid of interest are grown at 37.degree. C. in M9 plus
casamino acids medium with shaking in a air incubator Model G25 from New Brunswick Scientific
(Edison, N.J.). Growth is monitored at OD.sub.600 until it reaches a value of 1.0 at which time
Nalidixic acid (10 milligrams/mL) in 0.1 N NaOH is added to a final concentration of 50 .mu.g/mL.
The cultures are then shaken at 37.degree. C. for three to four additional hours. A high degree of
aeration is maintained throughout culture period in order to achieve maximal production of the
desired gene product. The cells are examined under a light microscope for the presence of inclusion
bodies (IB). One mL aliquots of the culture are removed for analysis of protein content by boiling
the pelleted cells, treating them with reducing buffer and electrophoresis via SDS-PAGE (see
Maniatis et al. Molecular Cloning: A Laboratory Manual, [1982]). After centrifugation (5000.times.g)
to pellet the cells, the first step in purification of the protein is either sonication or
homogenization of the cells. For sonication, the cells are resuspended in one-tenth volume (based on
culture size) sonication buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA). These resuspended cells are
subjected to several repeated sonication bursts using the microtip from a Sonicator cell disrupter,
Model W-375 obtained from Heat Systems-Ultrasonics Inc. (Farmingdale, N.Y.). The extent of
sonication is monitored by examining the homogenates under a light microscope. After all of the
cells are disrupted, the homogenates are fractionated by centrifugation at 10000.times.g for 20
minutes at 4.degree. C. in a JA-20 rotor and J2-21 centrifuge (Beckman, Fullerton, Calif.).
Alternatively, the IBs are released from the cells by lysing the cells in sonication buffer with a
Manton-Gaulin homogenizer (Holland) followed by centrifugation as above. The IB pellets, which are
highly enriched for the recombinant protein, are then subjected to another round of sonication and
centrifugation as described above. The recombinant protein is purified by a variety of standard
methods. The most common methods involve solubilization of the IBs with 4-6 molar urea or
guanidine-HCl buffers at pH 9-12, and air oxidation/folding in the presence of catalytic
concentrations of cysteine, beta-mercaptoethanol or dithiothreitol for 24 to 72 hours. The protein
is purified from E. coli contaminants using ion-exchange chromatography, such as Q-sepharose (anion)
and S-sepharose (cation), gel filtration, hydrophobic chromatography or reversed phase HPLC. After
dialysis against a low ionic strength buffer, the purified protein is stored frozen or lyophilized.

7. Document ID: US 6214586 B1

L11: Entry 7 of 28
File: USPT
Apr 10, 2001

US-PAT-NO: 6214586
DOCUMENT-IDENTIFIER: US 6214586 B1
TITLE: Method for purifying plasmid DNA and plasmid DNA substantially free of genomic DNA
DATE-ISSUED: April 10, 2001

US-CL-CURRENT: 435/91.1; 435/252.8, 435/259, 536/25.4, 536/25.41

APPL-NO: 8/ 986885
DATE FILED: December 8, 1997

IN: McNeilly; David S.

AB: A method is described for purifying plasmid DNA from a mixture containing plasmid
DNA and genomic DNA. A solution containing both plasmid DNA and genomic DNA is treated with at
least 80% by weight saturation ammonium sulfate, thereby precipitating the genomic DNA and
providing purified plasmid DNA in solution. Genomic DNA levels in the purified plasmid DNA
product are less than 1% by weight based on the plasmid DNA. The purified plasmid DNA is
suitable for use in humans.

L11: Entry 7 of 28
File: USPT
Apr 10, 2001

DOCUMENT-IDENTIFIER: US 6214586 B1
TITLE: Method for purifying plasmid DNA and plasmid DNA substantially free of genomic DNA

CLPR:
9. The method according to claim 4, further comprising treating the supernatant by reverse phase
chromatography to obtain an eluant containing purified plasmid DNA.

CLPR:
15. The method according to claim 11, further comprising treating the supernatant by reverse phase
chromatography to obtain an eluant containing purified plasmid DNA.

8. Document ID: US 6197553 B1

L11: Entry 8 of 28

File: USPT

Mar 6, 2001

US-PAT-NO: 6197553
DOCUMENT-IDENTIFIER: US 6197553 B1
TITLE: Method for large scale plasmid purification
DATE-ISSUED: March 6, 2001

US-CL-CURRENT: 435/91.1; 424/184.1, 435/259, 435/306.1, 435/320.1, 514/44, 536/23.1, 536/25.4

APPL-NO: 8/ 952428
DATE FILED: November 7, 1997

PARENT-CASE:
RELATED APPLICATION This is a 35 U.S.C. .sctn.371 U.S. national application of PCT/US96/07083, filed
May 15, 1996, which is a continuation-in-part of U.S. application Ser. No. 08/446,118, filed May 19,
1995, now abandoned, which is a continuation-in-part of U.S. application Ser. No. 08/275,571, filed
Jul. 15, 1994, now abandoned.

IN: Lee; Ann L, Sagar; Sangeetha

AB: A process is disclosed for the large scale isolation and purification of plasmid DNA
from large scale microbial fermentations. The process exploits a rapid heating method to induce
cell lysis and precipitate genomic DNA, proteins and other debris while keeping the plasmid in
solution. Suspending the microbial cells in buffer and then heating the suspension to about
70-100.degree. C. in a flow-through heat exchanger results in excellent lysis. Continuous flow
or batch-wise centrifugation of the lysate effects a pellet that contains the cell debris,
protein and most of the genomic DNA while the plasmid remains in the supernatant. This
invention offers a number of advantages including higher product recovery than by chemical
lyses, inactivation of Dnases, operational simplicity and scaleability.

L11: Entry 8 of 28

File: USPT

Mar 6, 2001

DOCUMENT-IDENTIFIER: US 6197553 B1
TITLE: Method for large scale plasmid purification

DEPR:
Isolation of the plasmid DNA from harvested microbial cells using the current lab scale procedures
consist mainly of enzymatic treatment of microbial cells to weaken the cell wall followed by cell
lysis. The purification steps include repetitive CsCl/EtBr centrifugations followed by organic
solvent extractions and precipitation to remove tRNA, residual proteins, EtBr and other host
contaminants. These steps are not scaleable and therefore not suitable for use in large-scale
processing. In contrast, preparative scale chromatography is a powerful purification tool that
provides high resolution, operational ease and increased productivity for purifying DNA plasmid
products. Two different modes of chromatography, reversed phase and anion exchange, show suitability
in purifying DNA plasmid to the stringent levels required for human use. Separations based on
reversed phase are governed by hydrophobic interactions while those for anion exchange are based on
electrostatic interaction. These two orthogonal chromatography steps achieve separations between
various forms of plasmid (supercoiled, open relaxed, linear and concatemers) and remove host
contaminants like LPS (endotoxin), RNA, DNA and residual proteins.

DEPR:
The anion exchange product was then loaded onto a reversed phase chromatography column (Poros R/H)
which had been previously equilibrated with 100 mM ammonium bicarbonate at pH 8.0, and a gradient of
0% to 80% methanol was used to elute the bound material. The highly purified supercoiled plasmid DNA
eluted at 22% methanol.

9. Document ID: US 6190901 B1

L11: Entry 9 of 28

File: USPT

Feb 20, 2001

US-PAT-NO: 6190901
DOCUMENT-IDENTIFIER: US 6190901 B1
TITLE: Chicken interleukin-15 and uses thereof
DATE-ISSUED: February 20, 2001

US-CL-CURRENT: 435/252.3; 435/320.1, 530/351, 536/23.5

APPL-NO: 8/ 729004
DATE FILED: October 10, 1996

PARENT-CASE:
This application claims the benefit of U.S. Provisional Application No. 60/005,682, filed Oct. 17,
1995.

IN: Sundick; Roy S., Jones; Lily A., Smith; David I.

AB: The present invention pertains to isolated DNA encoding avian interleukin-15 and to
purified interleukin-15 polypeptides.

L11: Entry 9 of 28

File: USPT

Feb 20, 2001

DOCUMENT-IDENTIFIER: US 6190901 B1
TITLE: Chicken interleukin-15 and uses thereof

DEPR:

Purification of IL-15 from natural or recombinant sources may be achieved by methods well-known in the art, including without limitation ion-exchange chromatography, reverse-phase chromatography on C4 columns, gel filtration, isoelectric focusing, affinity chromatography, immunoaffinity chromatography, and the like. In a preferred embodiment, large quantities of bioactive IL-15 may be obtained by constructing a recombinant DNA sequence comprising the coding region for IL-15 fused in frame to a sequence encoding 6 C-terminal histidine residues in the pSFV1 replicon (GIBCO/BRL). mRNA encoded by this plasmid is synthesized using techniques well-known to those skilled in the art and introduced into BHK-21 cells by electroporation. The cells synthesize and secrete mature glycosylated IL-15 polypeptides containing 6 C-terminal histidines. The modified IL-15 polypeptides are easily purified from the cell supernatant by affinity chromatography using a histidine-binding resin (His-bind, Novagen, Madison, Wis.).

10. Document ID: US 6168918 B1

L11: Entry 10 of 28

File: USPT

Jan 2, 2001

US-PAT-NO: 6168918
DOCUMENT-IDENTIFIER: US 6168918 B1
TITLE: Method of detecting foreign DNA integrated in eukaryotic chromosomes
DATE-ISSUED: January 2, 2001

US-CL-CURRENT: 435/6; 435/912, 536/23.1

APPL-NO: 8/ 594141
DATE FILED: January 31, 1996

IN: Satishchandran; C., Ciccarelli; Richard Benjamin, Pachuk; Catherine Julia

AB: Methods of detecting the presence of a plasmid DNA sequence integrated in a chromosomal DNA molecule of a eukaryotic cell in a sample that contains chromosomal DNA molecules of eukaryotic cells and free plasmid DNA molecules are disclosed. According to the invention, chromosomal DNA of eukaryotic cells which are free of deoxyadenosine methyltransferase, and free plasmid DNA molecules which are produced in cells that contain deoxyadenosine methyltransferase and which have a DpnI site, are digested with one or more restriction enzymes that cleave plasmid DNA sequences integrated in the chromosomal DNA and plasmid DNA molecules to produce DNA digestion segments that are then fractionated to produce a plurality of fractions. The DNA digestion segments in each fraction is digested with DpnI and plasmid DNA sequences are amplified using sets of primers that flank a DpnI site in the plasmid DNA sequence. The presence of amplified fragments indicates the presence of a plasmid DNA sequence integrated into the chromosomal DNA.

L11: Entry 10 of 28

File: USPT

Jan 2, 2001

DOCUMENT-IDENTIFIER: US 6168918 B1
TITLE: Method of detecting foreign DNA integrated in eukaryotic chromosomes

DEPR:
Following digestion, the DNA digestion segments are fractionated to produce a plurality of fractions of DNA digestion segments. The DNA is physically fractionated using any of a variety of known methods, e.g., gel filtration which fractionates on the basis of size, capillary electrophoresis which fractionates on the basis of charge, gel electrophoresis which fractionates on the basis of size and charge, anion exchange chromatography which fractionates on the basis of negative charges, reverse phase chromatography which fractionates on the basis of hydrophobicity and ion pair chromatography which fractionates on the basis of charge and hydrophobicity, or sucrose or other gradient methods which fractionate on the basis of density. In a preferred embodiment, the DNA digestion segments are fractionated by a gel filtration column chromatographic method, using, e.g., Sephacryl.TM. S1000. The physical fractionation separates the various DNA fragments into a range of sizes. The gel filtration method results in an elution distribution wherein the large fragments come off first whereas the smaller fragments are held up longer and come off the column later. The large chromosomal fragments (.about.100 kb) elute first, there is a peak of mid-size fragments (.about.30-40 kb), and the small plasmid fragments, from both free plasmid DNA molecules and potentially integrated plasmid DNA sequences (.about.2 kb or less), are eluted last. These fragment sizes will of course vary depending on the known plasmid sequence, the host genome, and the number and nature of the restriction enzymes selected. DNA digestion segments may be fractionated by any of several well known fractionation criteria such as size, charge, size and charge, hydrophobicity and density using well known and routine methods. Size fractionation may be done by size exclusion chromatography. Charge fractionation may be done by capillary electrophoresis. Size and charge fractionation may be done by agarose gel electrophoresis.

11. Document ID: US 6001653 A

L11: Entry 11 of 28

File: USPT

Dec 14, 1999

US-PAT-NO: 6001653
DOCUMENT-IDENTIFIER: US 6001653 A
TITLE: Human type 2 RNase H
DATE-ISSUED: December 14, 1999

US-CL-CURRENT: 435/375; 435/193, 435/320.1, 435/325, 435/440, 435/455, 435/6, 435/91.1, 536/23.1, 536/23.2, 536/24.3, 536/24.31, 536/24.33, 536/24.5, 536/25.3

APPL-NO: 9/ 203716
DATE FILED: December 2, 1998

PARENT-CASE:
This application claims the benefit of U.S. Provisional Application 60/067,458, filed Dec. 4, 1997.

IN: Crooke; Stanley T., Lima; Walter F., Wu; Hongjiang

AB: The present invention provides polynucleotides and polypeptides encoded thereby of human Type 2 RNase H. Methods of using these polynucleotides and polypeptides in enhancing antisense oligonucleotide therapies are also provided.

L11: Entry 11 of 28

File: USPT

Dec 14, 1999

DOCUMENT-IDENTIFIER: US 6001653 A
TITLE: Human type 2 RNase H

DEPR:
The cDNA fragment coding the full RNase H protein sequence was amplified by PCR using 2 primers, one of which contains restriction enzyme NdeI site adapter and six histidine (his-tag) codons and 22 bp protein N terminal coding sequence. The fragment was cloned into expression vector pET17b (Novagen, Madison, Wis.) and confirmed by DNA sequencing. The plasmid was transfected into E. coli BL21(DE3) (Novagen, Madison, Wis.). The bacteria were grown in M9ZB medium at 32.degree. C. and harvested when the OD.sub.600 of the culture reached 0.8, in accordance with procedures described by Ausubel et al., Current Protocols in Molecular Biology, Wiley and Sons, New York, N.Y., 1988. Cells were lysed in 8M urea solution and recombinant protein was partially purified with Ni-NTA agarose (Qiagen, Germany). Further purification was performed with C4 reverse phase chromatography (Beckman, System Gold, Fullerton, Calif.) with 0.1% TFA water and 0.1% TFA acetonitrile gradient of 0% to 80% in 40 minutes as described by Deutscher, M. P., Guide to Protein Purification, Methods in Enzymology 182, Academic Press, New York, N.Y., 1990. The recombinant proteins and control samples were collected, lyophilized and subjected to 12% SDS-PAGE as described by Ausubel et al. (1988) Current Protocols in Molecular Biology, Wiley and Sons, New York, N.Y. The purified protein and control samples were resuspended in 6 M urea solution containing 20 mM Tris HCl, pH 7.4, 400 mM NaCl, 20% glycerol, 0.2 mM PMSF, 5 mM DTT, 10 .mu.g/ml aprotinin and leupeptin, and refolded by dialysis with decreasing urea concentration from 6 M to 0.5 M as well as DTT concentration from 5 mM to 0.5 mM as described by Deutscher, M. P., Guide to Protein Purification, Methods in Enzymology 182, Academic Press, New York, N.Y., 1990. The refolded proteins were concentrated (10 fold) by Centricon (Amicon, Danvers, Mass.) and subjected to RNase H activity assay.

US-CL-CURRENT: 435/53; 435/189, 435/190, 435/252.3, 435/68.1

APPL-NO: 8/645193
DATE FILED: May 13, 1996

IN: Kupke; Thomas, Gotz; Friedrich, Kempter; Christoph, Jung; Gunther

AB: A method is described for oxidatively decarboxylating a peptide, comprising combining a peptide with EpiD, wherein the peptide comprises at its carboxy terminus the amino acid sequence X.sub.1 X.sub.2 X.sub.3 X.sub.4 X.sub.5 X.sub.6 C, wherein X.sub.1, X.sub.2, X.sub.3 and X.sub.4 are any one of the twenty common amino acids, X.sub.5 is Tyr, Val, Met, Leu, Ile, Phe or Trp, and X.sub.6 is Cys, Ala, Ser, Val, or Thr, whereby the oxidative decarboxylation of the peptide occurs.

L11: Entry 12 of 28

File: USPT

Oct 5, 1999

DOCUMENT-IDENTIFIER: US 5962253 A
TITLE: Oxidative decarboxylation of peptides catalyzed by flavoprotein EpiD

DEPR:
Purification of EpiD and Enzyme Assay--EpiD was purified as described previously using E. coli expression plasmid pT7-5apiD (Kupke, T. et al, J. Bacteriol. 174:5354-5361 (1992)). The concentration of the EpiD solution was determined according to Bradford (Bradford, M. M., Anal. Biochem. 72:248-254 (1976)); the concentrations of the precursor peptide solutions were estimated by the absorbance at 205 nm using an extinction coefficient of 31 for a protein solution of 1 mg/ml (Janson, J. C., & Ryden, L., Methods Enzymol. 193:441-455 (1990)); the synthetic peptic substrates were weighed out. The enzyme was assayed for 0.5-1 h at 37.degree. C. in 20 mM Tris/HCl buffer (pH 8.0) containing 3 mM dithiothreitol, 1-2 .mu.g of EpiD, and 10-25 .mu.g of precursor peptide or 50 .mu.g of synthetic peptide in a total volume of 1 ml. The reaction mixture was then separated by reversed phase chromatography using a .mu.RPC C2/C18 SC 2.1/10 column (Pharmacia Biotech Inc.). Peptides were eluted with a linear gradient of 0-50% acetonitrile, 0.1% trifluoroacetic acid in 3.8 ml with a flow rate of 200 .mu.l/min. The absorbance was measured simultaneously at 214, 260, and 280 nm. The peptides were collected by the peak fractionation method, dried with a vacuum concentrator, stored at -70.degree. C., dissolved in 30% acetonitrile, and analyzed by ES-MS.

12. Document ID: US 5962253 A

L11: Entry 12 of 28

File: USPT

Oct 5, 1999

US-PAT-NO: 5962253
DOCUMENT-IDENTIFIER: US 5962253 A
TITLE: Oxidative decarboxylation of peptides catalyzed by flavoprotein EpiD
DATE-ISSUED: October 5, 1999

13. Document ID: US 5726040 A

L11: Entry 13 of 28

File: USPT

Mar 10, 1998

US-PAT-NO: 5726040
DOCUMENT-IDENTIFIER: US 5726040 A
TITLE: Cosmetic compositions including tropoelastin isomorphs

DATE-ISSUED: March 10, 1998

US-CL-CURRENT: 435/69.1; 424/401, 424/59, 424/69, 424/78.03, 435/252.3, 435/252.33, 435/320.1, 435/325, 435/366, 435/69.7, 435/69.8, 514/12, 514/2, 530/350, 530/353

APPL-NO: 8/ 641627
DATE FILED: May 2, 1996

PARENT-CASE:
This is a continuation of Ser. No. 08/455,647 (now abandoned) filed May 31, 1995 which is a continuation Ser. No. 08/150,712 (now abandoned), filed on Nov. 10, 1993.

IN: Ensley; Burt D., Ludmer; Matthew

AB: A cosmetic composition including a non naturally-occurring extracellular matrix protein in combination with a cosmetic carrier is described. The protein is preferably of human origin and has not been previously cross-linked. The protein is most preferably selected from the group consisting of soluble human procollagen and soluble human tropoelastin. The protein may include at least one additional non-naturally occurring amino acid sequence moiety, the amino acid sequence moiety selected from the group consisting of a hydrophobic sequence, a hydrophilic sequence, and a lysine-rich sequence., A cosmetic composition in which the non naturally-occurring extracellular matrix protein is an isomorphic form of the protein is also described. The isomorph of the protein is preferably selected from the group consisting of elastin isomorphs, collagen isomorphs and fibronectin isomorphs. Most preferably, the isomorphs are of human origin and have not been previously cross-linked., Methods of making and using a cosmetic composition of the invention are also described.

L11: Entry 13 of 28

File: USPT

Mar 10, 1998

DOCUMENT-IDENTIFIER: US 5726040 A
TITLE: Cosmetic compositions including tropoelastin isomorphs

DEPR:
The elastin cDNA, containing the synthetic oligonucleotides in reading frame, cloned into, for example, pAS-MCS72, is transformed into the lysogenic host E. coli AR120, and transformants selected using routine procedures. Bacteria bearing the expression plasmid induced by, for example, 60 mg/ml nalidixic acid, are shaken at 37.degree. C. to allow for expression of the tropoelastin isomorph. Bacterial pellets are suspended in buffer, treated with lysozyme and then centrifuged. The pellet from the lysozyme treatment is suspended in buffer, homogenized, and centrifuged. The pellets, containing tropoelastin associated with the cell membranes, is treated with CNBr, releasing solubilized, intact tropoelastin fusion protein. Additional purification is achieved using reverse phase chromatography, or other method.

14. Document ID: US 5707812 A

L11: Entry 14 of 28

File: USPT

Jan 13, 1998

US-PAT-NO: 5707812
DOCUMENT-IDENTIFIER: US 5707812 A
TITLE: Purification of plasmid DNA during column chromatography
DATE-ISSUED: January 13, 1998

US-CL-CURRENT: 435/6; 435/252.3, 435/320.1, 536/23.1

APPL-NO: 8/ 692590
DATE FILED: August 6, 1996

IN: Horn; Nancy, Budahazi; Greg, Marquet; Magda

AB: A method for purifying plasmid DNA during column chromatography is provided. A short chain polymeric alcohol, preferably polyethylene glycol, or another DNA condensation agent, is added to a DNA sample prior to column chromatography. The short chain polymeric alcohol or condensation agent promotes improved isolation of plasmid DNA and may be used for large scale purification, particularly for manufacturing plasmid DNA as a biopharmaceutical.

L11: Entry 14 of 28

File: USPT

Jan 13, 1998

DOCUMENT-IDENTIFIER: US 5707812 A
TITLE: Purification of plasmid DNA during column chromatography

BSPR:
The use of short chain polymeric alcohols, like polyethylene glycol, and other condensation agents that cause plasmid DNA to act homogeneously for purposes of purification is not limited to ion exchange chromatography. It extends to other chromatographic methods, including size exclusion chromatography, chromatofocusing, affinity chromatography, hydrophobic interaction chromatography, and reversed phase chromatography. Indeed, this use extends broadly to other purification methods, e.g., diafiltration, ultrafiltration, and filtration generally, in which the isolation of plasmid DNA from RNA, proteins and other contaminants is facilitated by causing various plasmid DNA species to act as a class.

15. Document ID: US 5614379 A

L11: Entry 15 of 28

File: USPT

Mar 25, 1997

US-PAT-NO: 5614379
DOCUMENT-IDENTIFIER: US 5614379 A
TITLE: Process for preparing anti-obesity protein
DATE-ISSUED: March 25, 1997

US-CL-CURRENT: 435/68.1; 435/212

APPL-NO: 8/ 429362

DATE FILED: April 26, 1995

IN: MacKellar; Warren C.

AB: The present invention is directed to a novel process of preparing an anti-obesity protein using dipeptidyl-aminopeptidase isolated from the cellular slime mold, Dictyostelium discodeum. The process produces an anti-obesity protein in high yield.

L11: Entry 15 of 28

File: USPT

Mar 25, 1997

DOCUMENT-IDENTIFIER: US 5614379 A
TITLE: Process for preparing anti-obesity protein

DEPR:
SEQ ID NO: 3 was produced as an insoluble aggregate in the cytoplasm of E-coli. that carried a plasmid which encoded the above-mentioned protein. The insoluble protein was solubilized in 8M urea.
The conversion reaction was initiated by the addition of 3-6 milliunits dDAP per mg SEQ ID NO: 3.
The conversion reaction was allowed to proceed for 6-8 hours at room temperature. Reaction rates car be increased by adding more enzyme increasing the concentration of SEQ ID NO: 3 or increasing the reaction temperature. The progress of the reaction was monitored by high performance reversed phase chromatography. The reaction was terminated by adjusting the pH to 8 with NaOH. The converted des(Met-Arg)SEQ ID NO: 3 was further purified by anion exchange and size exclusion chromatography.
After conversion analytical procedures including peptide mapping, N-terminal sequencing, mass spectroscopy and reversed phase HPLC indicated that the met-arg on the N-terminus was cleaved.

molecule and a target is provided. A plurality of beads is applied to a substrate, each bead having associated therewith multiple copies of only a single oligomeric molecule. The beads are substantially spaced-apart from one another and are substantially immobilized on the substrate. Conditions are applied such that a substantial portion of the multiple copies associated with each bead can disassociate from and diffuse into the substrate, the substrate constructed and arranged to permit only substantially localized diffusion. A localized signal occurring as a result of the diffusion is detected. Coated beads and libraries of coated beads also are provided.

L11: Entry 16 of 28

File: USPT

Feb 11, 1997

DOCUMENT-IDENTIFIER: US 5601992 A
TITLE: Peptide library formats and methods relating thereto

DEPR:
To confirm that the identified peptides were bombesin receptor agonists, they were resynthesized by standard means and dose response curves were obtained against melanophores expressing recombinant receptors as shown in FIG. 5. In particular, peptides were synthesized on Rink Amide MBHA resin (0.25 mmol scale) using Fmoc chemistry and were purified using HPLC and reverse phase chromatography on a C18 column. In FIG. 6, the abilities of the peptides to effect circle growth, expressed as the slopes of the lines in FIG. 5, are compared with their measured EC.sub.50 values. The dose responses curves were obtained by microtiter plate assays using the melanophores cells transfected with pJG3.6BR plasmid DNA.

16. Document ID: US 5601992 A

L11: Entry 16 of 28

File: USPT

Feb 11, 1997

US-PAT-NO: 5601992
DOCUMENT-IDENTIFIER: US 5601992 A
TITLE: Peptide library formats and methods relating thereto
DATE-ISSUED: February 11, 1997

US-CL-CURRENT: 435/7.2; 435/7.1, 435/7.21, 435/7.23, 436/501

APPL-NO: 8/ 303585
DATE FILED: September 9, 1994

PARENT-CASE:
RELATED APPLICATIONS This application is a continuation of Ser. No. 08/068,904, filed May 28, 1993 now abandoned, which is a continuation-in-part of U.S. application Ser. No. 07/917,502, filed Jul. 21, 1992 now abandoned, the entire disclosure of which is incorporated herein by reference.

IN: Lerner; Michael R., Jayawickreme; Channa K., Lerner; Ethan A.

AB: A method for detecting the interaction between an oligomeric

17. Document ID: US 5596072 A

L11: Entry 17 of 28

File: USPT

Jan 21, 1997

US-PAT-NO: 5596072
DOCUMENT-IDENTIFIER: US 5596072 A
TITLE: Method of refolding human IL-13
DATE-ISSUED: January 21, 1997

US-CL-CURRENT: 530/351; 424/85.2, 435/69.1, 530/402, 530/412, 930/141

APPL-NO: 8/ 012543
DATE FILED: February 1, 1993

PARENT-CASE:
This application is a continuation-in-part of commonly assigned patent application U.S. Ser. No. 07/933,416, filed on Aug. 21, 1992, now abandoned, which is incorporated herein by reference.

IN: Culpepper; Janice, McKenzie; Andrew, Dang; Warren, Zurawski; Gerard

AB: Nucleic acids encoding human IL-13, and purified IL-13

proteins and fragments
thereof. Antibodies, both polyclonal and monoclonal, are also provided. Methods of using the
compositions for both diagnostic and therapeutic utilities are provided.

L11: Entry 17 of 28

File: USPT

Jan 21, 1997

DOCUMENT-IDENTIFIER: US 5596072 A
TITLE: Method of refolding human IL-13

DEPR:
E. coli-derived hIL-4 (van Kimmenade et al. (1988) Eur. J. BioChem. 173:109-114), human
interleukin-1.alpha. (hIL-1.alpha.; Kronheim et al. (1986) Bio/Technology 4:1078-1082), and mIL-13
(see above) were purified as described. hIL-4Y124D was prepared from E. coli K12 cells (strain CQ21)
harboring the DTrpC11-hIL-4.Y142D plasmid grown overnight at 37.degree. C. in 12 liters of L-Broth
containing 50 .mu.g/ml ampicillin in a G 53 rotatory shaker (New Brunswick Scientific) at 200 rpm.
The cells were harvested by centrifugation in a RC-3 centrifuge (all rotors Sorvall) at 4,500 rpm,
10 min, 4.degree. C. The pellets were resuspended in 450 ml of TE buffer (50 mM Tris-HCl pH 8, 1 mM
EDTA) by shaking at 200 rpm for 15 min. Cells were ruptured by 4 passes through an ice-cooled
Microfluidizer model 110 cell disrupter (Microfluidics). Inclusion bodies were collected by
centrifugation in a GS-3 rotor at 9,000 rpm, 40 min, 4.degree. C. The pellet was then washed by
resuspension in 450 ml of TE and Triton X-100 was added to a final concentration of 0.5%. Samples
were kept at room temperature for 30 min and were then pelleted in a GSA rotor at 8,500 rpm, 10 min,
4.degree. C. The inclusion bodies were resuspended in 60 ml 5M guanidine-HCl in PBS (120 mM NaCl,
2.7 mM KCl, 10 mM NaPi pH 7.4), 2 mM reduced glutathione, 0.2 mM oxidized glutathione and any
remaining insoluble material was removed by centrifugation in a SS-34 rotor at 20,000 rpm, 30 min,
4.degree. C. The supernatant was diluted 10-fold into the same buffer without guanidine
hydrochloride and stirred gently overnight at 4.degree. C. to permit refolding and oxidization.
Concentration and exchange into 100 ml 50 mMNa Acetate pH 5.0 was then performed using a Millipore
Pellicon apparatus (Millipore) equipped with a tangential flow ultrafiltration cassette with a size
exclusion of 10 kDa. The sample was subjected to anion exchange chromatography (CM sepharose 16/100
column, Pharmacia) in the same buffer with elution via a 0-0.7M NaCl gradient. Fractions containing
hIL-4 protein were pooled and subjected to reverse phase chromatography (Poros R 10/100 column,
Perseptive Biosystems) with elution via a gradient of 0-50% acetonitrile in 0.1% trifluoroacetic
acid/water. Fractions containing hIL-4 were lyophilized, dissolved in 50 mMNa Acetate pH 5.0, and
quantified by densitometry (Molecular Dynamics) of stained SDS-PAGE with chicken egg lysozyme
(Sigma) as a standard.

18. Document ID: US 5593877 A

L11: Entry 18 of 28

File: USPT

Jan 14, 1997

US-PAT-NO: 5593877
DOCUMENT-IDENTIFIER: US 5593877 A
TITLE: Nucleic acid and recombinant production of vespid venom hyaluronidase
DATE-ISSUED: January 14, 1997

US-CL-CURRENT: 435/197; 435/320.1, 435/69.1, 536/23.2, 536/23.5, 536/24.31

APPL-NO: 8/ 180209
DATE FILED: January 11, 1994

PARENT-CASE:
The present invention is a continuation-in part of application Ser. No. 08/031,400, filed Mar. 11,
1993, now abandoned, of which the instant Application claims the benefit of the filing date under 35
U.S.C. .sctn.120, and the disclosure of which is incorporated herein by reference in its entirety.

IN: King; Te P.

AB: The present invention is directed to nucleic acids encoding vespid venom enzymes, or
fragments thereof, recombinant vectors comprising such nucleic acids, and host cells containing
the recombinant vectors. The invention is further directed to expression of such nucleic acids
to produce recombinant vespid venom enzymes, or recombinant fragments, derivatives or analogs
thereof. Such recombinant products are useful for diagnosis of allergy and for therapeutic
treatment of allergy. In specific embodiments, the present invention provides nucleic acids
encoding, and complete nucleotide and amino acids sequences for, vespid venom phospholipase,
for example, Dolichovespula maculata phospholipase and Vespula vulgaris phospholipase, and
vespid venom hyaluronidase, for example, Dolichovespula maculata hyaluronidase.

L11: Entry 18 of 28

File: USPT

Jan 14, 1997

DOCUMENT-IDENTIFIER: US 5593877 A
TITLE: Nucleic acid and recombinant production of vespid venom hyaluronidase

DEPR:
The pQE12 plasmid is designed so that the recombinant protein has the sequence:
MRGS-insert-SRH.sub.6. The presence of the hexa-histidine sequence in the recombinant protein makes
possible its purification from other bacterial proteins by metal ion chelation chromatography
followed by reversed phase chromatography.

19. Document ID: US 5583031 A

L11: Entry 19 of 28

File: USPT

Dec 10, 1996

US-PAT-NO: 5583031
DOCUMENT-IDENTIFIER: US 5583031 A
TITLE: Empty major histocompatibility class II heterodimers
DATE-ISSUED: December 10, 1996

US-CL-CURRENT: 435/69.1; 424/184.1, 424/185.1, 424/193.1, 435/320.1, 435/348, 435/69.3, 514/8, 530/395, 530/403, 530/868

APPL-NO: 7/ 831895
DATE FILED: February 6, 1992

IN: Stern; Lawrence J.

AB: The invention features an isolated sample of mammalian class II major histocompatibility heterodimers which are membrane-associated or in soluble form, and which are capable of binding added antigenic peptide; methods for producing large amounts of the soluble or membrane-associated histocompatibility protein by expression of DNA encoding the .alpha. and .beta. polypeptides; and methods for loading these heterodimers with any desired antigen.

L11: Entry 19 of 28
File: USPT
Dec 10, 1996

DOCUMENT-IDENTIFIER: US 5583031 A
TITLE: Empty major histocompatibility class II heterodimers

DEPR:
Oligonucleotides were synthesized with a Milligen model 3700 DNA synthesizer using .beta.-cyanoethyl phosphoroamidite chemistry, and were purified by denaturing acrylamide gel electrophoresis and reverse-phase chromatography on Sep-pack (Millipore) cartridges. Baculovirus transfer plasmids pVL1393 and pAC360-.beta.gal and the wild-type baculovirus ACMNPV-E2 are available from In Vitrogen. Restriction enzymes and DNA modifying enzymes were obtained from New England Biolabs, Boehringer Mannheim, US Biochemicals and Promega.

20. Document ID: US 5561064 A

L11: Entry 20 of 28
File: USPT
Oct 1, 1996

US-PAT-NO: 5561064
DOCUMENT-IDENTIFIER: US 5561064 A
TITLE: Production of pharmaceutical-grade plasmid DNA
DATE-ISSUED: October 1, 1996

US-CL-CURRENT: 435/320.1; 435/259, 435/91.1

APPL-NO: 8/ 192151
DATE FILED: February 1, 1994

IN: Marquet; Magda, Horn; Nancy, Meek; Jennifer, Budahazi; Gregg

AB: The invention relates to a method for producing plasmid DNA, comprising the steps of: (a) lysing cells containing the plasmid DNA to obtain a lysate; (b) treating the lysate by a means for removing insoluble material to obtain a solute; and (c) applying the solute to differential PEG precipitations and chromatography to purify the plasmid DNA. In other embodiments of the invention, the plasmid DNA is produced with GRAS reagents; the plasmid DNA is produced in the absence of enzymes; the plasmid DNA is produced in the absence of organic extractants; the plasmid DNA is produced in the absence of mutagens; the lysing, treating and applying steps are scalable to result in the large scale manufacture of the plasmid DNA; and the lysing, treating and applying steps result in the generation of pharmaceutical grade material.

L11: Entry 20 of 28
File: USPT
Oct 1, 1996

DOCUMENT-IDENTIFIER: US 5561064 A
TITLE: Production of pharmaceutical-grade plasmid DNA

DEPR:
Upon being separated from many host contaminants, such as chromosomal DNA, RNA, lipopolysaccharide and protein, a sample is obtained that is rich in plasmid DNA and yet may harbor small RNA oligonucleotides, trace amounts of chromosomal DNA, protein, endotoxins and residues left over from processing. According to the invention, further purification may be effected as an independent step to rid product of remaining nucleic acids, macromolecules, small molecular forms and residuals, and, moreover, to isolate covalently closed circular DNA, i.e., supercoiled monomers, from nicked circular plasmids (relaxed monomers) and concatenated forms (supercoiled dimers, etc.). Towards this end, a chromatography step is performed. Differences in ionic charge, molecular size, and/or other characteristics are exploited to bring about purification of the desired plasmid DNA species. Chromatography is contemplated to encompass ion exchange chromatography, size exclusion chromatography, reversed phase chromatography, hydrophobicity interaction chromatography, affinity chromatography, and any like chromatography, and, also, any combination of these to bring about the final purification of plasmid DNA.

21. Document ID: US 5247070 A

L11: Entry 21 of 28
File: USPT
Sep 21, 1993

US-PAT-NO: 5247070
DOCUMENT-IDENTIFIER: US 5247070 A
TITLE: N-terminal muteins of tumor necrosis factor
DATE-ISSUED: September 21, 1993

US-CL-CURRENT: 530/351; 424/85.1, 435/69.5, 435/69.7, 530/395, 530/402, 930/144

APPL-NO: 7/ 763512
DATE FILED: September 20, 1991

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY
APPL-NO
APPL-DATE
JP
2-250045
September 21, 1990
JP
3-240130
June 17, 1991

IN: Yamada; Nobutoshi, Kato; Masanari, Miyata; Keizo, Aoyama; Yoshiyuki, Shikama; Hiroshi

AB: A polypeptide which is a tumor necrosis factor polypeptide having an amino acid sequence represented by from the 1st Ser to the 155th Leu as shown by SEQ ID NO:1 in the Sequence Listing, or its mutein, wherein the amino acid sequence of the 1st Ser to the 8th Asp of the SEQ ID NO:1 or the corresponding amino acid sequence of the mutein is replaced by an amino acid sequence containing at least one amino acid sequence of cell-adherent peptide of laminin and from 5 to 30 amino acids. Also disclosed are a recombinant plasmid containing a DNA encoding such a polypeptide, a recombinant microbial cell transformed by such a recombinant plasmid, a process for producing the polypeptide, a pharmaceutical composition comprising the polypeptide as an active ingredient, and a DNA for the polypeptide.

L11: Entry 21 of 28
File: USPT
Sep 21, 1993

DOCUMENT-IDENTIFIER: US 5247070 A
TITLE: N-terminal muteins of tumor necrosis factor

DEPR:
Synthesized oligonucleotides are then purified by a conventional purification method, such as high performance chromatography using a reversed phase chromatography column, or electrophoresis, using polyacrylamide gel. Thereafter, the oligonucleotides are phosphorylated by means of, e.g., T4 polynucleotide kinase, then annealed and ligated by means of T4 DNA ligase. Here, the oligonucleotides are divided into several blocks and sequentially ligated so that the human TNF gene sequence will eventually be obtained, followed by digestion with restriction endonucleases or polishing (make blunt-end) with T4 DNA polymerase, and the resulting DNA fragments are then purified by electrophoresis. The obtained DNA fragments are inserted into plasmid vectors such as pUC8, pUC9, pUC18 and pUC19 (J. Messing et al., Gene, 19, 259 (1982)), and the inserted plasmid vectors are introduced into competent cells for cloning in accordance with a usual method. From the obtained clones, plasmid DNAs are extracted and purified by a usual method, and they are examined to see whether or not the nucleotide sequences of the DNA fragments inserted into the vectors agree with the desired gene sequence. The respective sections of the human TNF gene thus obtained, are then cut out from the plasmid vectors containing them, by means of restriction endonucleases, then ligated and inserted again into the above vector to obtain a plasmid vector having the desired full length of the human TNF gene. The plasmid vector thus obtained is digested by restriction endonucleases and separated and purified by gel electrophoresis to obtain the desired human TNF gene.

22. Document ID: US 5242808 A

L11: Entry 22 of 28
File: USPT
Sep 7, 1993

US-PAT-NO: 5242808
DOCUMENT-IDENTIFIER: US 5242808 A
TITLE: Production of bioadhesive precursor protein analogs by genetically engineered organisms
DATE-ISSUED: September 7, 1993

US-CL-CURRENT: 435/69.1; 435/252.3, 435/252.33, 435/254.21, 435/254.3, 435/320.1, 435/471, 435/69.7, 530/353

DISCLAIMER DATE: 20080917
APPL-NO: 7/ 644745
DATE FILED: January 23, 1991

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application is a continuation of application Ser. No. 07/025,243, filed Mar. 12, 1987, abandoned, which is a continuation-in-part of application Ser. No. 06/933,945, filed Nov. 24, 1986, abandoned, which is a continuation-in-part of application Ser. No. 06/650,128, filed Sep. 13, 1984, abandoned.

IN: Maugh; Kathy J., Anderson; David M., Strausberg; Susan L., Strausberg; Robert, Wei; Tena

AB: Recombinant production of bioadhesive precursor protein analogs is disclosed. The bioadhesive precursor protein analogs can be hydroxylated and used as an adhesive in wet environments.

L11: Entry 22 of 28
File: USPT
Sep 7, 1993

DOCUMENT-IDENTIFIER: US 5242808 A
TITLE: Production of bioadhesive precursor protein analogs by genetically engineered organisms

DEPL:
After purification by preparative gel electrophoresis and reverse-phase chromatography, the oligonucleotides were dissolved at a concentration of 1 delta 280 unit/ml. Oligonucleotides #1876, #1988, and #1892 were phosphorylated individually in reactions with T.sub.4 polynucleotide kinase and 1 mM ATP with 20 ul of oligonucleotide solution added in a 50 ul kinase reaction.
Oligonucleotides #1545 and #1546 were similarly treated, except they were pooled first at a 1:1 ratio. After the enzyme reaction, the solutions were boiled for two minutes to inactivate the enzyme. An equivalent amount of oligonucleotide #1875 was added to the #1876 kinase reaction, boiled for 30 seconds, then allowed to slow cool for formation of 5' linker Likewise, the #1892 and #1877

kinase reactions were mixed together with an equivalent amount of non-kinased #1893, boiled, slow cooled and then ligated in a 180 ul volume at 16.degree. C. for 11 hours with T.sub.4 polynucleotide ligase to assemble the 3' linker. Plasmid pGX2287 DNA (5 ug) was digested with 18 units of ClaI endonuclease then extracted with phenol-chloroform, ethanol precipitated and dissolved in 0.01 M Tris-HCl, 0.001 M EDTA (pH 8.0) at 0.25 ug DNA/ul. Ten microliters of the ClaI-cut pGX2287 DNA was ligated with 25 ul of the 5' linker in a total volume of 40 ul at 16.degree. C. for 11 hours. After ligation, the DNA was phenol-chloroform extracted, ethanol precipitated, then dissolved in 1 ml water. The DNA solution was concentrated using a Centricon 30 (Amicon) ultrafiltration unit, then washed two times with 2 ml water and centrifuged at 5,000 RPM for ten minutes. The washed and concentrated DNA, largely free of non-ligated linkers, was ethanol precipitated and dissolved in 10 microliters of water.

23. Document ID: US 5217593 A

L11: Entry 23 of 28

File: USPT

Jun 8, 1993

US-PAT-NO: 5217593
DOCUMENT-IDENTIFIER: US 5217593 A
TITLE: Nucleic acid purification system and method
DATE-ISSUED: June 8, 1993

US-CL-CURRENT: 204/457; 204/608

APPL-NO: 7/911515
DATE FILED: July 10, 1992

PARENT-CASE:
REFERENCE TO RELATED APPLICATION The present application is a continuation-in-part of co-pending application Ser. No. 07/668,856, now U.S. Pat. No. 5,139,637 filed Mar. 13, 1991 and entitled PLASMID PURIFICATION SYSTEM AND METHOD.

IN: MacConnell; William P.

AB: A process for the purification of DNA and the like comprises a housing having walls forming a reservoir having a chamber for containing a buffer solution, means for circulating a buffer through the reservoir, a disposable cassette within said chamber having first means including a gel for defining a first path extending between an inlet end and an outlet end, a well for introducing a bacterial sample into the path at said inlet end thereof, and a second path intersecting the first path via an elution chamber, having a collection chamber including an elution window at said outlet end, and an electrical circuit for selectively applying an electrical potential along each of the paths for selectively moving a plasmid first along the first path from the bacterial well to the elution chamber, then along the second path to the collection window at the end thereof.

L11: Entry 23 of 28

File: USPT

Jun 8, 1993

DOCUMENT-IDENTIFIER: US 5217593 A
TITLE: Nucleic acid purification system and method

BSPR:
Many techniques and apparatus exist for small scale purification of plasmid DNA. The typical prior art approach to the purification of plasmids involves a series of steps, including a collection of cells grown in liquid culture by centrifugation, separation of the bacterial chromatic (genomic) DNA, and cellular debris from the soluble contents of the bacteria by centrifugation of filtration, and concentration of the plasmid DNA apart from other cellular components by alcohol or isopropanol, absorption to solid media (i.e. ion exchange resin, glass powder, reverse phase chromatography resin, etc.), or salt precipitation. Additional purification steps may be added to these, such as phenol/chloroform extraction, secondary alcohol precipitation, protease or ribonuclease treatment to further purity the plasmid DNA.

24. Document ID: US 5139637 A

L11: Entry 24 of 28

File: USPT

Aug 18, 1992

US-PAT-NO: 5139637
DOCUMENT-IDENTIFIER: US 5139637 A
TITLE: Plasmid purification system and method
DATE-ISSUED: August 18, 1992

US-CL-CURRENT: 204/466; 204/616

APPL-NO: 7/668856
DATE FILED: March 13, 1991

IN: MacConnell; William P.

AB: An apparatus for the purification of DNA and the like comprises a housing having walls forming a reservoir having a plurality of chambers for containing a buffer solution means for circulating a buffer through the reservoir, a disposable cassette within said housing having first means including a gel for defining a first path extending between a first pair of the chambers, a well for introducing a bacterial sample into the path at one end thereof, and a second path intersecting the first path via an elution window at one end, having a collection window at the other end and extending between a second pair of the chambers, and an electrical circuit for selectively applying an electrical potential along each of the paths for selectively moving a plasmid first along the first path from the bacterial well to the elution window, then along the second path to the collection window at the end thereof.

L11: Entry 24 of 28

File: USPT

Aug 18, 1992

DOCUMENT-IDENTIFIER: US 5139637 A
TITLE: Plasmid purification system and method

BSPR:
Many techniques and apparatus exist for small scale purification of plasmid DNA. The typical prior art approach to the purification of plasmids involves a series of steps, including a collection of cells grown in liquid culture by centrifugation, separation of the bacterial chromatic (genomic) DNA, and cellular debris from the soluble contents of the bacteria by centrifugation of filtration, and concentration of the plasmid DNA apart from other cellular components by alcohol or isopropanol, absorption to solid media (i.e. ion exchange resin, glass powder, reverse phase chromatography resin, etc.), or salt precipitation. Additional purification steps may be added to these, such as phenol/chloroform extraction, secondary alcohol precipitation, protease or ribonuclease treatment to further purity the plasmid DNA.

25. Document ID: US 4699717 A

L11: Entry 25 of 28

File: USPT

Oct 13, 1987

US-PAT-NO: 4699717
DOCUMENT-IDENTIFIER: US 4699717 A
TITLE: Chromatographic process for the separation of nucleic acids
DATE-ISSUED: October 13, 1987

US-CL-CURRENT: 536/25.4; 210/198.2, 210/502.1, 210/635, 210/656, 502/401, 502/439, 514/44, 536/26.73

APPL-NO: 6/ 830708
DATE FILED: February 14, 1986

PARENT-CASE:
This application is a continuation-in-part application of our copending application Ser. No. 560,931 filed Nov. 25, 1983, filed Mar. 25, 1983, published as WO83/03363 on Oct. 13, 1983, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY APPL-NO APPL-DATE
DE 3211309 March 26, 1982

IN: Riesner; Detlev, Colpan; Metin

AB: A process for the chromatographic separation of nucleic acid using a chromatographic carrier material is described in which the surface of the carrier material is specially modified.

L11: Entry 25 of 28

File: USPT

Oct 13, 1987

DOCUMENT-IDENTIFIER: US 4699717 A
TITLE: Chromatographic process for the separation of nucleic acids

BSPR:
The prior art ion exchangers and reversed-phase chromatography resins could only be used with high resolution for small molecules such as oligonucleotides, e.g. chain length up to 15 nucleotides (Fritz et al.; Biochemistry (1978) 17, 1257-1267). In the separation of nucleic acids of high molecular weight such as long-chain ribo- and deoxyribo-oligonucleotides, natural RNA's, like transfer RNA and 7S RNA, viral RNA and messenger RNA, DNA, DNA fragments and plasmid DNA, the required resolution into individual nucleic acid species could not be obtained.

26. Document ID: JP 08163995 A

L11: Entry 26 of 28

File: JPAB

Jun 25, 1996

PUB-NO: JP408163995A
DOCUMENT-IDENTIFIER: JP 08163995 A
TITLE: PURIFYING METHOD OF VARIOUS BRAIN-DERIVED NEUROTROPHIC FACTOR AND VARIOUS BRAIN-DERIVED NEUROTROPHIC FACTOR

PUBN-DATE: June 25, 1996

INVENTOR-INFORMATION:
NAME COUNTRY
SHIMIZU, NORIO
FUKUZONO, SHINICHI

INT-CL (IPC): C12P 21/02; C07K 1/14; C07K 14/475; C07K 14/48; C12N 1/21; C12N 15/09

AB: PURPOSE: To obtain various brain-derived neurotrophic factors for a medicine, a diagnostic drug and a reagent, etc., having respectively different biological activity by centrifuging a host colibacillus-crushing solution producing a matured drain-derived neurotrophic factor, dividing into a supernatant and a guanidine hydrochloride-extracted solution of a precipitate, and purifying., CONSTITUTION: A gene-recombining colibacillus introduced of a recombining plasmid containing a gene coding for a matured brain-derived neurotrophic factor connected with a signal peptide gene of β-lactamase is cultured in a medium at 37°C for 24 hours, centrifuged and resultant fungi are gathered. The fungi are suspended in a phosphoric acid buffer solution and crushed in an ultrasonic crusher, and resultant solution of crushed fungus is centrifuged to divide into a supernatant and a precipitate. The centrifuged precipitate is extracted with a guanidine hydrochloride aqueous solution to divide into a centrifuged supernatant and a guanidine hydrochloride-extracted solution of the centrifuged precipitate. These solutions are purified by an ion-exchange chromatography, a gel filtration or a reverse phase chromatography to obtain the objective brain-derived neurotrophic factors such as a medicine, a diagnostic drug and a research reagent, etc., having respectively different biological activity.,
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L11: Entry 26 of 28

File: JPAB

Jun 25, 1996

DOCUMENT-IDENTIFIER: JP 08163995 A
TITLE: PURIFYING METHOD OF VARIOUS BRAIN-DERIVED NEUROTROPHIC FACTOR AND VARIOUS BRAIN-DERIVED NEUROTROPHIC FACTOR

FPAR:
CONSTITUTION: A gene-recombining colibacillus introduced of a recombining plasmid containing a gene coding for a matured brain-derived neurotrophic factor connected with a signal peptide gene of β-lactamase is cultured in a medium at 37°C for 24 hours, centrifuged and resultant fungi are gathered. The fungi are suspended in a phosphoric acid buffer solution and crushed in an ultrasonic crusher, and resultant solution of crushed fungus is centrifuged to divide into a supernatant and a precipitate. The centrifuged precipitate is extracted with a guanidine hydrochloride aqueous solution to divide into a centrifuged supernatant and a guanidine hydrochloride-extracted solution of the centrifuged precipitate. These solutions are purified by an ion-exchange chromatography, a gel filtration or a reverse phase chromatography to obtain the objective brain-derived neurotrophic factors such as a medicine, a diagnostic drug and a research reagent, etc., having respectively different biological activity.

27. Document ID: JP 08163995 A

L11: Entry 27 of 28

File: DWPI

Jun 25, 1996

DERWENT-ACC-NO: 1996-348981
DERWENT-WEEK: 199635
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TITLE: Purification of brain-derived nerve nutrition factor used e.g. in drugs - from supernatant obtd. by disruption and centrifugation of genetic recombinant E. coli

PRIORITY-DATA: 1994JP-0312940 (December 16, 1994)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 08163995 A	June 25, 1996	N/A	006	C12P021/02

INT-CL (IPC): C07K 1/14; C07K 14/475; C07K 14/48; C12N 1/21; C12N 15/09; C12P 21/02; C12P 21/02; C12R 1/19; C12N 1/21; C12R 1/19

AB: Claimed is a method of purifying mature brain-derived nerve nutrition factor (BDNF) from a supernatant obtained by disruption and centrifugation of genetic recombinant E. coli producing BDNF. Also claimed in a method of purifying BDNF extracted with guanidine hydrochloride from precipitates obtained by disruption and centrifugation of genetic recombinant E. coli producing BDNF., The purificn. includes ion-exchange chromatography, gel filtration and reverse phase chromatography. The BDNF-producing E. coli carries a plasmid contg. a BDNF gene linked to a single peptide gene for beta-lactamase. Disulphide linkages in BDNF purified from recombinant E. coli are e.g. Cys(5)-Cys(68), Cys(80)-Cys(109), Cys(13)-Cys(111)., USE/ADVANTAGE - BDNF is used as drugs, diagnostic drugs, reagents for research, etc. Disrupted microorganisms from which BDNF is obtained is divided into a supernatant obtained by centrifugation and an extract obtained with guanidine hydrochloride from a pellet of the centrifuged microorganisms, and the supernatant gives wild-type BDNF and the extract gives 3 types of BDNF having biological activities (in terms of ED50) of 2, 9 and 15 ng/ml respectively. Use of a signal sequence from E. coli permits BDNF to be produced in periplasm of E. coli.

L11: Entry 27 of 28

File: DWPI

Jun 25, 1996

DERWENT-ACC-NO: 1996-348981
DERWENT-WEEK: 199635
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TITLE: Purification of brain-derived nerve nutrition factor used e.g. in drugs - from supernatant obtd. by disruption and centrifugation of genetic recombinant E. coli

ABTX:
The purificn. includes ion-exchange chromatography, gel filtration and reverse phase chromatography.
The BDNF-producing E. coli carries a plasmid contg. a BDNF gene linked to a single peptide gene for beta-lactamase. Disulphide linkages in BDNF purified from recombinant E. coli are e.g. Cys(5)-Cys(68), Cys(80)-Cys(109), Cys(13)-Cys(111).

28. Document ID: JP 04234988 A

L11: Entry 28 of 28

File: DWPI

Aug 24, 1992

DERWENT-ACC-NO: 1992-327632
DERWENT-WEEK: 199240
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TITLE: Human prolactin and its protein A fused protein expression gene - for treatment and diagnosis of pituitary tumours and sterility

PRIORITY-DATA: 1991JP-0012506 (January 11, 1991)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE

PAGES

JP 04234988 A MAIN-IPC

August 24, 1992

N/A

008

C12N015/62

INT-CL (IPC): A61B 10/00; A61K 37/32; C07K 13/00; C12N 15/62; C12P 21/02; G01N 33/53; C12P 21/02; C12R 1/19

AB: Fused protein expression gene has a gene coding collagenase (III) cleavage site of amino acid sequence (I) (Gly-Xaa-Gly-Pro-Xaa) between human prolactin (I) gene and a gene coding the region (PA) contg. at least Fc-bond active peptide fragment of a protein A (II)., USE/ADVANTAGE - Used in pharmaceuticals for diagnosis of pituitary tumour and sterility., In an example, cDNA of (I) is prepd. (I) gene is cloned. One of 6 clones is digested by EcoRI and recombined to the EcoRI site of plasmid vector pUC9 to give cDNA cloning vector of (I). It is introduced to E. coli and amplified. DNA coding (III) linker is prepd. PA-(III) linker-(I)-fused protein expression vector is constructed. PA(III) linker-(I)-fused protein expression vector, pRIT-LK-PL1.2, is expressed. (I) is purified from the fused protein by a Sepharose 4B column, a HPLC and a reversed phase chromatography. The cleavage condition of the pure fused protein by (III) is examined. (I) shows a same immunological reactivity as commercial (I). It shows no crossing over with other hormones such as human growth hormone and human placental lactogen as to an antibody prepd. by using the fused protein as the antigen

L11: Entry 28 of 28

File: DWPI

Aug 24, 1992

DERWENT-ACC-NO: 1992-327632
DERWENT-WEEK: 199240
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TITLE: Human prolactin and its protein A fused protein expression gene - for treatment and diagnosis of pituitary tumours and sterility

ABTX:
In an example, cDNA of (I) is prepd. (I) gene is cloned. One of 6 clones is digested by EcoRI and recombined to the EcoRI site of plasmid vector pUC9 to give cDNA cloning vector of (I). It is introduced to E. coli and amplified. DNA coding (III) linker is prepd. PA-(III) linker-(I)-fused protein expression vector is constructed. PA(III) linker-(I)-fused protein expression vector, pRIT-LK-PL1.2, is expressed. (I) is purified from the fused protein by a Sepharose 4B column, a HPLC and a reversed phase chromatography. The cleavage condition of the pure fused protein by (III) is examined. (I) shows a same immunological reactivity as commercial (I). It shows no crossing over with other hormones such as human growth hormone and human placental lactogen as to an antibody prepd. by using the fused protein as the antigen